

# **Binuclear DOTA-based Gd(III) chelates: Revisiting a straightforward strategy for relaxivity improvement**

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## Abstract

The need for MRI contrast agents with improved relaxivity maintains the development of new Gd(III) chelates an intensive and demanding field of research. In this work we introduce the new dimeric chelators bis(DOTA-AHA)adipate and bis(DOTA-AHA)1,3-phenyldiacetate (L2 = bis(1,4,7,10-tetraazacyclododecane-1-((6-amino)hexanoic)-4,7,10-triacetic acid)adipate and L3 = bis(1,4,7,10-tetraazacyclododecane-1-((6-amino)hexanoic)-4,7,10-triacetic acid)1,3-phenyldiacetate, respectively), based on the bifunctional ligand DOTA-AHA (L1 = 1,4,7,10-tetraazacyclododecane-1-((6-amino)hexanoic)-4,7,10-triacetic acid). Their Gd(III) chelates were studied by variable temperature  $^1\text{H}$  NMRD and  $^{17}\text{O}$  NMR spectroscopy in order to measure the relaxivity and the parameters that govern it. The exchange of inner-sphere water from the monomer GdL1 and from the two binuclear chelates Gd<sub>2</sub>L2 and Gd<sub>2</sub>L3 is very similar ( $^{298}k_{\text{ex}} \approx 6.5 \times 10^6 \text{ s}^{-1}$ ) and slightly faster than on  $[\text{Gd}(\text{DOTA})\text{H}_2\text{O}]^-$  ( $^{298}k_{\text{ex}} = 4.1 \times 10^6 \text{ s}^{-1}$ ). All three compounds form weakly bound aggregates with equilibrium constants  $^{298}K$  of 2.9, 15.6 and 14.6 for GdL1, Gd<sub>2</sub>L2 and Gd<sub>2</sub>L3, respectively. Even if the aggregates contain only 10 to 15% of the total amount of Gd(III) ions a marked increase in relaxivity between 30 and 100 MHz is observed.

Furthermore the distance between the two Gd(III) centers in the binuclear compounds has been determined by double electron-electron resonance (DEER) experiments and by molecular modelling studies affording comparable distances. The linkers between the chelating moieties allow Gd(III)-Gd(III) distances of *circa* 3.0 nm for completely stretched linker conformation and  $\leq 1.9$  nm for the conformation with the metal centers at closer distance. These metal to metal distances by themselves cannot explain the considerably long tumbling times of chelates in solution. Only a model consistent with some level of aggregation for the binuclear chelates in aqueous solution could satisfactorily explain our results.

## Introduction

Magnetic resonance imaging (MRI) has become one of the most powerful and useful techniques in medicine for soft tissue imaging. This imaging technique is based on the interaction of magnetic fields and radiofrequencies with the water molecules of the

body.<sup>[1]</sup> The quality of a MRI scan depends on intrinsic properties of the biological tissues such as the density ( $\rho_H$ ) of the hydrogen nuclei, the blood flow and the hydrogen nuclei relaxation times ( $T_1$  and  $T_2$ ).<sup>[2]</sup> However, soft tissues have little difference in proton density (not more than 10%) and consequently image intensity often displays low contrast.<sup>[1]</sup> In such cases there is the need of contrast agents (CAs) to further enhance the contrast between the tissues. These CAs are paramagnetic compounds which shorten the relaxation times of local proton spins ( $T_1$  and  $T_2$ ), increasing the signal intensity on  $T_1$  weighted images and decreasing it on  $T_2$  weighted images.<sup>[3]</sup>

The majority of the approved CAs are gadolinium chelates based both on macrocyclic tetraazapolyaminocarboxylate chelators (ex: Dotarem<sup>®</sup>, Prohance<sup>®</sup> or Gadovist<sup>®</sup>) and open-chain polyaminocarboxylate chelators (ex: Magnevist<sup>®</sup>, Omniscan<sup>®</sup> or Multihance<sup>®</sup>). These low molecular weight chelates constitute the first generation of MRI contrast agents and are extracellular fluid agents since they equilibrate rapidly between the intravascular and interstitial space and do not interact specifically with any type of cells.<sup>[4]</sup> The effectiveness of any contrast agent is measured by its relaxivity ( $r$ ), which is the enhancement of the water protons relaxation rate imposed by a 1 mM concentration of Gd(III) chelate.<sup>[5]</sup> The development of low molecular weight CAs with enhanced relaxivities is highly desirable and several small Gd(III) chelates have been reported over the past years.<sup>[6]</sup>

There are several approaches to increase the relaxivity through the optimization of its molecular parameters. The rotational correlation time ( $\tau_R$ ), water exchange rate ( $k_{ex}$ ) and electron spin relaxation times ( $T_e$ ) are the most important parameters determining relaxivity. Because  $T_e$  is mainly dependent on the metal ion it is not easily changeable, but the other two parameters can be tuned by ligand design.<sup>[5]</sup> However, the optimization of both parameters via chelator design is challenging and demanding.<sup>[7]</sup> The development of molecular constructs containing several gadolinium units is a strategy that has been applied often. It relies on the formation of multinuclear assemblies, either through covalently bound chelates such as multimeric structures,<sup>[8]</sup> linear polymeric structures<sup>[8d, 9]</sup> or spherical dendrimers;<sup>[10]</sup> or non-covalently bound chelates such as micelles,<sup>[11]</sup> liposomes,<sup>[11c, 12]</sup> or even metal ion-assisted aggregation of gadolinium chelates.<sup>[13]</sup> All these molecular constructs present longer rotational correlational times. To tune this parameter seems to be the most simple and

straightforward choice, since  $\tau_R$  values have a direct correlation with the weight and size of the chelate.<sup>[14]</sup>

Chemical modifications in the chelating moiety should not interfere with the ability to retain Gd(III), since it has been reported severe toxicity associated with repeated CAs in individuals suffering from kidney function impairment.<sup>[15]</sup> The design of new gadolinium chelates that ensure the patient's safety associated with the possibility of relaxivity improvement has become the prime goal in the development of new CAs, and it favors the use of macrocyclic DOTA-type ligands.<sup>[16]</sup> Over the past years, several bifunctional DOTA-based chelators bearing extra functional groups in one acetate arm have been reported in the literature.<sup>[17]</sup> The extra functional group in the pendant arm allows the binding of the Gd(III) chelate to a plethora of chemical moieties through different linkages without reducing the thermodynamic stability and kinetic inertness.

Accordingly, in the first part of this paper we report the synthesis of a new bifunctional chelator, 1,4,7,10-tetraazacyclododecane-1-((6-amino)hexanoic)-(4,7,10-triacetic acid (DOTA-AHA) (Figure 1 – L1). Although its gadolinium chelate Gd(DOTA-AHA) may be considered a candidate for contrast agent, it is nevertheless more promising as a building block for multinuclear chelates of concomitant higher relaxivities.

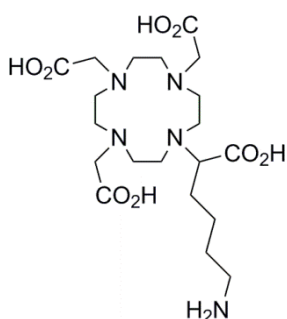
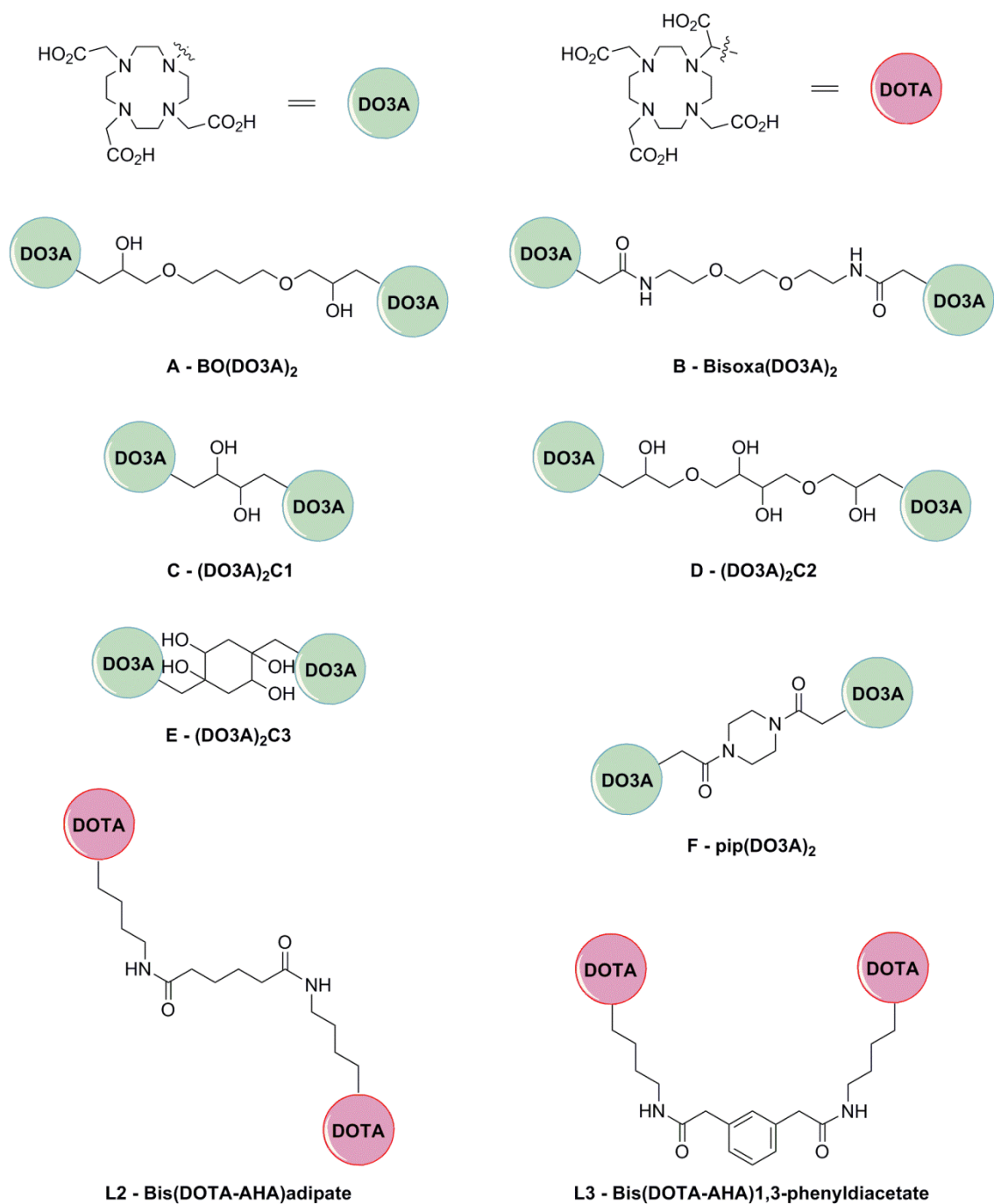


Figure 1. DOTA-AHA (L1) bifunctional ligand.

Despite showing improvement in relaxivity, large Gd(III)-containing molecular constructs also show some drawbacks. Their elevated size is responsible for the slow leakage from blood vessels to interstitial space through the normal endothelium of vascular systems.<sup>[4]</sup> This phenomenon provides long imaging windows, but limits their applicability. Their pharmacokinetic profile is also a concern, since these molecules may also be important “targets” for enzymatic systems, which may result in undesired metabolism. Moreover, problems with the excretion of these macromolecular Gd(III) agents are recurrent, since the glomerular filtration can decrease drastically.<sup>[18]</sup>

Alternatively, dimerization can be a straightforward way to obtain CAs with improved relaxivity, without drastically increasing the molecular weight and size.<sup>[14, 19]</sup> Several DO3A-based binuclear Gd(III)<sup>[20]</sup> (Scheme 1 – A to F) and other Ln(III)<sup>[21]</sup> chelates are found in the literature. Overall, the gadolinium chelates present improved relaxivity per metal ion in comparison to Gd(DOTA)<sup>-</sup>, due to optimization of the rotational correlational time. Simultaneously, the dimerization of these Gd(DO3A)-based chelates leads to neutral charged complexes opposing to the electrocharged monomers from which they were synthesized. Negatively charged chelates show higher  $^{298}k_{\text{ex}}$  values than the corresponding electroneutral ones.<sup>[6b]</sup> Therefore, the relaxivity values potentially achievable by chelates displaying relatively long  $\tau_R$  values are not reached due to the limited water exchange rates of these electroneutral Gd(DO3A)-binuclear chelates. To overcome this downside, we synthesized two DOTA-AHA-based dimeric ligands, the bis(1,4,7,10-tetraazacyclododecane-1-((6-amino)hexanoic)-4,7,10-triacetic acid)adipate (bis(DOTA-AHA)adipate) and the bis(1,4,7,10-tetraazacyclododecane-1-((6-amino)hexanoic)-4,7,10-triacetic acid)1,3-phenyldiacetate (bis(DOTA-AHA)1,3-phenyldiacetate) (Scheme 1 – L2 and L3). Their Gd(III) chelates have been studied by <sup>1</sup>H NMRD, <sup>17</sup>ONMR and EPR and the results obtained are reported in this paper.



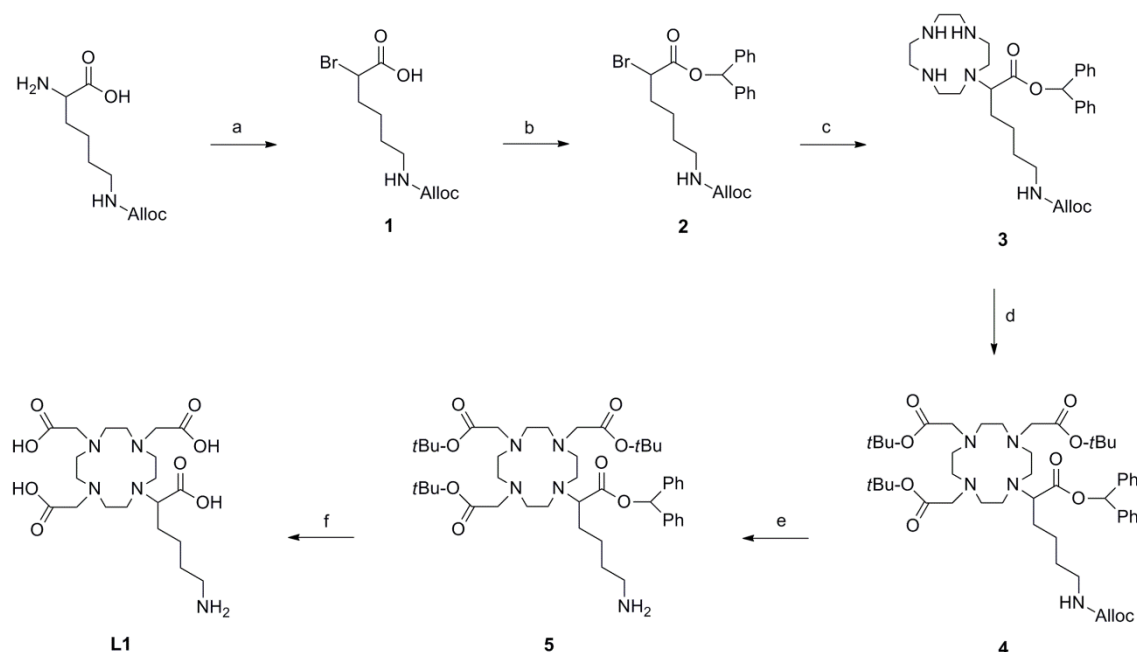
Scheme 1. DO3A and DOTA-based dimeric ligands for Gd(III). The DO3A ligands have been reported in the literature.<sup>[20]</sup>

## Results and Discussion

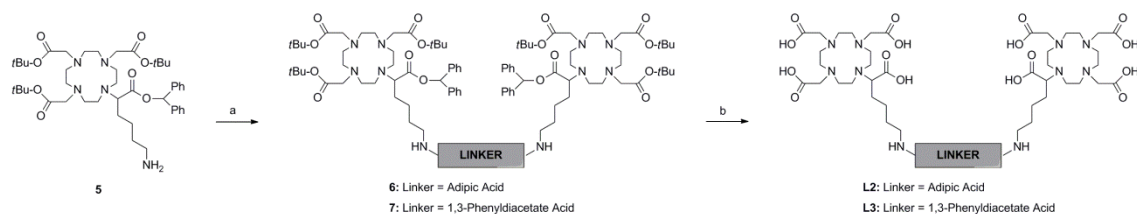
### Synthesis

The DOTA-AHA chelator was prepared from a lysine precursor using orthogonal protecting groups (scheme 2). The dimeric DOTA-AHA derivatives were obtained by reacting two dicarboxylic acids (adipic acid and 1,3-phenyldiacetic acid) with

compound **5** (scheme 3). After deprotection, the dimeric ligands bis(1,4,7,10-tetraazacyclododecane-1-((6-amino)hexanoic)-4,7,10-triacetic acid)adipate (bis(DOTA-AHA)adipate) and bis(1,4,7,10-tetraazacyclododecane-1-((6-amino)hexanoic)-4,7,10-triacetic acid)1,3-phenyldiacetate (bis(DOTA-AHA)1,3-phenyldiacetate)) were isolated.



Scheme 2. Synthetic pathway of DOTA-AHA chelator (L1). a) NaBr, NaNO<sub>2</sub>, HBr (1 M); b) DDM, Me<sub>2</sub>CO; c) Cyclen, K<sub>2</sub>CO<sub>3</sub>, MeCN; d) *tert*-butyl bromoacetate, K<sub>2</sub>CO<sub>3</sub>, MeCN; e) Pd(PPh<sub>3</sub>)<sub>4</sub>, Me<sub>2</sub>NH.BH<sub>3</sub>, DCM; f) TFA, DCM.



Scheme 3. Synthetic scheme of DOTA-AHA dimers. a) adipic acid (**6**) or 1,3-phenyldiacetic acid (**7**), DIPEA, HATU, HOBt, MeCN; b) TFA, DCM.

## <sup>1</sup>H NMRD and <sup>17</sup>O NMR Relaxometric Studies

To obtain parameters characterizing the efficiency in view of water relaxation enhancement of the mononuclear compound GdL1, as well as the binuclear compounds Gd<sub>2</sub>L2 and Gd<sub>2</sub>L3, <sup>1</sup>H NMRD profiles and <sup>17</sup>O relaxation and chemical shift measurements have been performed (Figure 2). From the experimental NMRD data it can be seen that the two binuclear compounds clearly show a relaxivity hump between

20 and 120 MHz whereas the relaxivity profile of the mononuclear compound is rather flat (Figure 2c). The reduced transverse  $^{17}\text{O}$  relaxation,  $1/T_{2r}$ , is very similar for the three compounds over the whole temperature range studied (Figure 2a), indicating that water exchange should be similar. The reduced longitudinal  $^{17}\text{O}$  relaxation,  $1/T_{1r}$ , is also similar which would indicate that the rotational motion of the mononuclear and binuclear compounds do not differ significantly. Apparently this is in contradiction to the NMRD data.

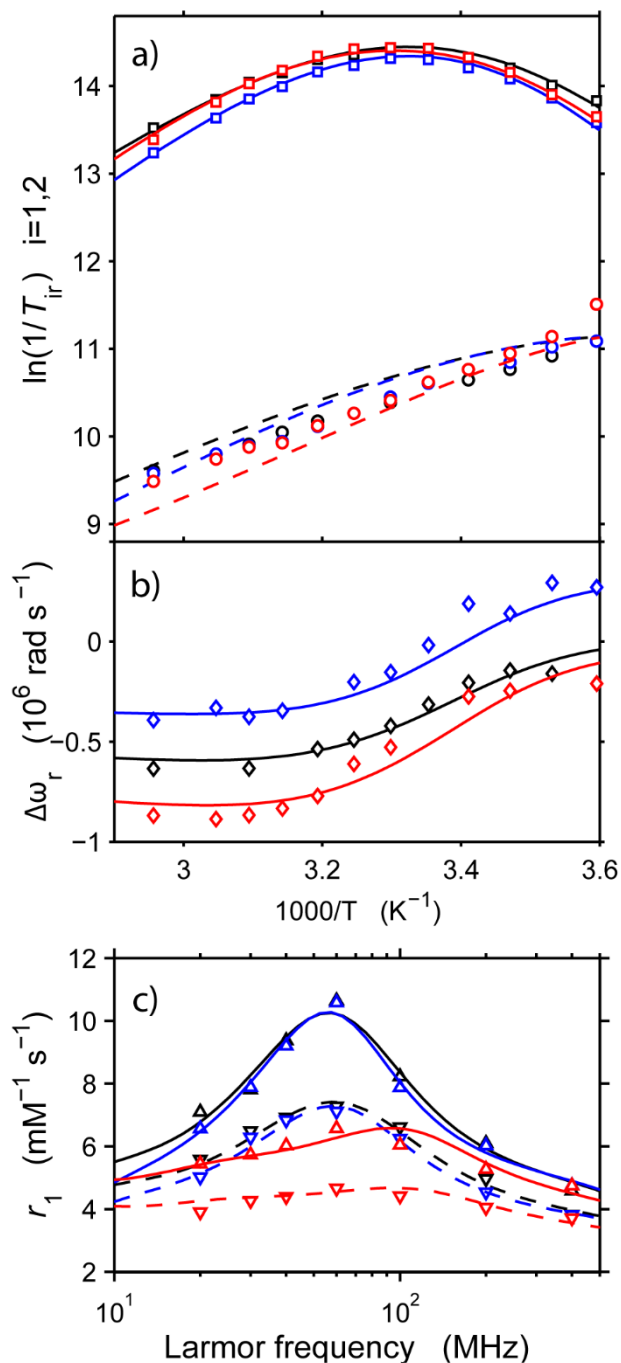


Figure 2. (a) - Reduced transverse (squares) and longitudinal (circles)  $^{17}\text{O}$  NMR relaxation rates for GdL1 (20 mM [Gd(III)] (red), Gd<sub>2</sub>L2 (black) (12 mM [Gd(III)]) and Gd<sub>2</sub>L3 (blue) (12 mM [Gd(III)]); (b)



Reduced  $^{17}\text{O}$  chemical shifts for GdL1 ( $\diamond$ ), Gd<sub>2</sub>L2 ( $\diamond$ ) and Gd<sub>2</sub>L3 ( $\diamond$ ); (c)  $^1\text{H}$  NMRD profiles for GdL1 (red), Gd<sub>2</sub>L2 (black) and Gd<sub>2</sub>L3 (blue) at 25 °C ( $\triangle$ ) and 37 °C ( $\nabla$ ). The lines represent the best fit of the data resulting from simultaneous fitting based on SBM equations.

We assumed that standard Solomon-Bloembergen-Theory (SBM)<sup>[3-4]</sup> is valid in our case (see reference <sup>[7, 22]</sup> for a comparison of different theoretical approaches). The  $^1\text{H}$  NMRD and  $^{17}\text{O}$  NMR data have been evaluated using a simultaneous fitting procedure.<sup>[20b]</sup> This procedure is the most efficient way to get reliable parameters characterizing the relaxation properties of gadolinium complexes in solution:

- water exchange ( $k_{\text{ex}}$ ,  $\Delta H^\ddagger$ ) is best obtained from temperature dependent transverse  $^{17}\text{O}$  relaxation ( $1/T_{2r}$ ); the scalar coupling constant ( $A/\hbar$ ) entering this data can be obtained from  $^{17}\text{O}$  NMR chemical shift
- rotational correlation times ( $\tau_R$ ) are obtained from frequency dependent longitudinal  $^1\text{H}$  relaxation ( $r_1$ , NMRD) and from temperature dependent longitudinal  $^{17}\text{O}$  NMR relaxation ( $1/T_{1r}$ )

The information on the dynamics of the paramagnetic complexes is obtained by relaxation enhancement measured on bulk water molecules to which it has been transmitted by chemical exchange. It is not known *per se* if there is only one exchanging species present or if there are several with different rotational behavior.

A first attempt showed that the  $^1\text{H}$  NMRD and  $^{17}\text{O}$  NMR data could not be fitted satisfactorily using a single rotational correlation time (see Supporting Information). A satisfactory fit of the data could be obtained using two correlation times – a short one ( $\tau_R$ , < 200 ps) and a long one ( $\tau_R$ , > 2000 ps). In the case of Gd-complexes in solution there are two possible scenarios:

1. Most of the gadolinium complexes aggregate. The rotational diffusion of the Gd-H vectors can be described by the global reorientation of the aggregate (global correlation time  $\tau_g$ ) and by possible local motions (local correlation time  $\tau_l$ ). The theoretical description follows the Lipari-Szabo model developed for the dynamics of proteins.<sup>[23]</sup> The way the Gd-H vectors sense both motions is described by a model-free order parameter  $S$ .
2. Some gadolinium complexes aggregate but some are still present as single compounds. The rotational motion of the single compounds (monomers) is characterized by  $\tau_R^{\text{mono}}$  and the rotational motion of the aggregates by  $\tau_R^{\text{agg}}$ .

To check for aggregation of the compounds we measured  $^1\text{H}$  relaxivity as a function of concentration - in absence of aggregation the relaxivity,  $r_1$ , should not depend on  $[\text{Gd(III)}]$ . The results show that for both binuclear chelates  $r_1$  increases by more than 50% if the concentration of the paramagnetic ion is raised from 0.1 mM to about 12 mM (Figure 3). This increase is less than, for example, that observed for the trinuclear  $\text{Gd}_3\text{Ph}_4(\text{DTTA})_3$ <sup>[24]</sup> and it also starts at much higher concentration, 4 mM in contrast to 0.1 mM. The relaxivity of  $\text{Gd}_2\text{L2}$  and  $\text{Gd}_2\text{L3}$  measured at low concentration is close to that of the mononuclear compound  $\text{GdL1}$ . These data suggest that the binuclear compounds weakly aggregate. This finding is remarkable; first, there is no hydrophobic linker between the two chelates and second, the  $\text{Gd}(\text{DOTA})$ -chelates are charged and not neutral like the  $\text{Gd}(\text{DO3A})$ -type chelates.

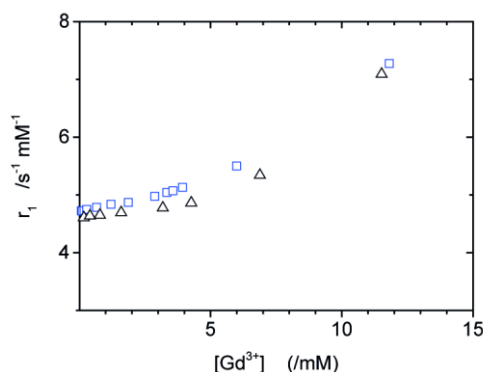


Figure 3. Concentration dependent relaxivity of the binuclear  $\text{Gd}_2\text{L2}$  (□) and  $\text{Gd}_2\text{L3}$  (△) measured at 1.41 T (60 MHz) and 37 °C.

The Lipari-Szabo model has been used to describe rotational diffusion of different kinds of compounds like dendrimers, polymers, micelles and aggregates. As far as we know the second scenario with weakly self-aggregating monomers has not yet been applied in the context of paramagnetic compounds forming weakly bound aggregates and exchanging water molecules with the bulk. To be able to treat the data with a reasonable number of parameters some assumptions have to be made:

First, the water exchange between the first coordination sphere and bulk does not change when the compounds form aggregates. This assumption is very reasonable because it has been shown that water exchange does not change markedly if binuclear compounds are formed using the same chelator.<sup>[25]</sup>

The second assumption is that all equilibrium constants for forming dimers, trimers, tetramers, etc. from the same monomer are equal. If we name the monomer A we have<sup>[26]</sup>



and

$$\begin{aligned}
 [A_2] &= K[A]^2 \\
 [A_3] &= K^2[A]^3 \\
 &\dots \\
 [A_n] &= K^{n-1}[A]^n
 \end{aligned}
 \tag{2}$$

The total amount of compound A in solution is given by<sup>[27]</sup>

$$\begin{aligned}
 [A]_0 &= [A] + 2[A_2] + 3[A_3] + \dots + n[A_n] \\
 &= [A] + 2K[A]^2 + 3K^2[A]^3 + \dots + nK^{n-1}[A]^n \\
 &= \frac{[A]}{(1 - K[A])^2}
 \end{aligned}
 \tag{3}$$

The temperature dependence of the equilibrium constant is

$$K = e^{-\left(\frac{\Delta H^0}{RT} - \frac{\Delta S^0}{R}\right)}
 \tag{4}$$

If the binding between the aggregate forming monomers is relatively weak we can assume that the rates of formation and dissociation of the aggregates are fast compared to the rate of water exchange. In this case we can calculate the enhancement of longitudinal relaxation  $1/T_{1r}$  as

$$\frac{1}{T_{1r}} = \frac{1}{\langle T_{1m} \rangle + \tau_m} \quad (5)$$

$$\text{with } \left\langle \frac{1}{T_{1m}} \right\rangle = \sum_{i=1}^n \frac{x_i}{T_{1m,i}} \quad (6)$$

where  $x_i$  are the mole fractions of  $\text{Gd}^{3+}$  in the monomer (1), dimer (2), etc and  $\langle T_{1m} \rangle$  is the weighted mean of the longitudinal relaxation rate of bound water spins. The mole fractions can be calculated from the equilibrium constants via the roots of equation 3. The relaxation rates of bound water molecules,  $1/T_{1m,i}$  in the different aggregates differ only by the rotational correlation times. Clearly, we should have  $\tau_{R,1} < \tau_{R,2} < \tau_{R,3} < \text{etc.}$  To keep the number of fitted parameters reasonable we used only two correlation times, that for the rotation of the monomer,  $\tau_{R,1} = \tau_R^{\text{mono}}$ , and a mean correlation time for the rotation of aggregates  $\langle \tau_{R,i} \rangle = \tau_R^{\text{agg}}$ . As a test we fitted the data of the binuclear compound  $\text{Gd}_2\text{L3}$  with three correlations times. The quality of the fit did not increase markedly and besides the correlation times all other parameters of the fits were unchanged (see Supporting Information).

Inspection of the equations resulting from the two scenarios described above shows that the theoretical descriptions are nearly equal with the main difference that the Lipari-Szabo order free parameter  $S^2$  is replaced by the mole fraction of  $\text{Gd(III)}$  present in the aggregates. In contrast to  $S^2$  the mole fraction depends on temperature leading to an increased quality of the fit (for details see Supporting Information). Consequently we fitted the three data sets using the self-aggregation model (Figure 2).

As can be seen from the results in Table 1 water exchange rate constants at room temperature,  $^{298}k_{\text{ex}}$ , are equal within experimental error. This is not surprising because all compounds use the same chelating unit to bind  $\text{Gd(III)}$ . The rate constants at 298 K are slightly higher than that of  $[\text{Gd}(\text{DOTA})]^-$  ( $^{298}k_{\text{ex}} = 4.1 \cdot 10^6 \text{ s}^{-1}$ ) but about four times faster than that of the DO3A based dimers  $[\text{bisoxa}[(\text{Gd}(\text{DO3A}))_2]]$  and  $[\text{pip}[(\text{Gd}(\text{DO3A}))_2]]$  ( $^{298}k_{\text{ex}} = 1.4 \cdot 10^6 \text{ s}^{-1}$  and  $^{298}k_{\text{ex}} = 1.5 \cdot 10^6 \text{ s}^{-1}$ , respectively). The increase in lability is most probably due to the negative charge of the DOTA compounds.<sup>[6b]</sup> From the positive entropies of activation,  $\Delta S^\ddagger$ , one can conclude that the mechanism has a more dissociative character than on DO3A-type complexes ( $\Delta S^\ddagger = +1.7$  and  $-11.7 \text{ J K}^{-1} \text{ mol}^{-1}$ ).

Table 1. Water exchange parameters,  $^{298}k_{\text{ex}}$ ,  $\Delta H^\ddagger$ ,  $\Delta S^\ddagger$ , rotational correlation times,  $^{298}\tau_{\text{R}}^{\text{mono}}$ ,  $^{298}\tau_{\text{R}}^{\text{agg}}$ , and equilibrium constants,  $K^{298}$ ,  $\Delta H^0$ ,  $\Delta S^0$ , for aggregation GdL1, Gd<sub>2</sub>L2 and Gd<sub>2</sub>L3. The parameters were obtained from a simultaneous fit of <sup>17</sup>O NMR and <sup>1</sup>H NMRD data using SBM theory.

	$^{298}k_{\text{ex}}$ (10 <sup>6</sup> s <sup>-1</sup> )	$\Delta H^\ddagger$ (kJ mol <sup>-1</sup> )	$\Delta S^\ddagger$ (J K <sup>-1</sup> mol <sup>-1</sup> )	$^{298}\tau_{\text{R}}^{\text{mono}}$ (ps)	$^{298}\tau_{\text{R}}^{\text{agg}}$ (ps)	$\Delta H^0$ (kJ mol <sup>-1</sup> )	$\Delta S^0$ (kJ mol <sup>-1</sup> )	$K^{298}$ (M <sup>-1</sup> )	$x_1^{\text{a)}$
<b>Mononuclear</b>									
GdL1	6.4 ± 1.4	58 ± 7	+81 ± 23	103 ± 13	2600 ± 2100	-41 ± 19	-129 ± 63	2.9	0.90 <sup>b)</sup>
<b>Binuclear</b>									
Gd <sub>2</sub> L2	6.8 ± 3	54 ± 8	+68 ± 30	129 ± 10	4000 ± 1800	-32 ± 9	-84 ± 31	15.6	0.85 <sup>c)</sup>
Gd <sub>2</sub> L3	6.4 ± 1.1	63 ± 5	+95 ± 18	134 ± 7	4000 ± 1200	-33 ± 6	-89 ± 20	14.6	0.86 <sup>c)</sup>

a) mole fraction of the monomer; b) at [GdL1] = 20 mM; c) at [Gd<sub>2</sub>L2/3] = 6 mM

Table 2. Distribution of gadolinium in monomers, dimers and trimers calculated from  $K^{298}$  and equation 3 at 298K.

	[Gd <sup>3+</sup> ]	$K^{298}$ (M <sup>-1</sup> )	monomer	dimer	trimer
<b>Mononuclear</b>					
GdL1	20 mM	2.9	89.9 %	9.4 %	0.7 %
<b>Binuclear</b>					
Gd <sub>2</sub> L2	12 mM	15.6	84.8 %	13.4 %	1.6
Gd <sub>2</sub> L3	12 mM	14.6	85.6%	12.8 %	1.4

All complexes, the mononuclear GdL1 as well as the binuclear Gd<sub>2</sub>L3 and Gd<sub>2</sub>L3 form aggregates in solution. For GdL1 the constant for complex formation  $K^{298}$  is 5 to 6 times smaller than for the binuclear complexes (Table 1). From the equilibrium constants we can calculate the relative amounts of Gd(III) ions as monomer, dimer or trimer (Table 2). For the mononuclear species dimers are present only in a small amount ( $\approx 10\%$ ). For both binuclear species the amount of Gd(III) present in an aggregate is  $\approx 15\%$  and the major aggregated form is again the dimer. The intermolecular interaction leading to aggregation is much weaker between our compounds if compared to DO3A based binuclear compounds.<sup>[28]</sup> The seven-coordinating DO3A chelator allows formation of carboxylate bridges and leads therefore to very stable aggregates.

From the point of view of water proton relaxivity the compounds in its monomeric or aggregated form are characterized by the rotational correlation times  $\tau_R^{\text{mono}}$  and  $\tau_R^{\text{agg}}$ , respectively. The values obtained by the fitting differ for all three compounds by more than one order of magnitude (Table 1). Surprisingly the  $^{298}\tau_R^{\text{mono}}$  value fitted for the mononuclear compound is only 25% shorter than the corresponding values of the binuclear compounds. A possible explanation could be a high degree of internal rotation in both binuclear compounds. The longer value of  $^{298}\tau_R^{\text{mono}}$  of GdL1 in respect to for example Gd(DOTA) can be explained by the C<sub>5</sub>NH<sub>2</sub>-chain attached to the ligand. The  $^{298}\tau_R^{\text{mono}}$  of the binuclear Gd<sub>2</sub>L2 and Gd<sub>2</sub>L3 are in the same order as those of other binuclear compounds (Gd<sub>2</sub>(bisoxa(DO3A))<sub>2</sub>: 106 ps,<sup>[20b]</sup> Gd<sub>2</sub>(pip(DO3A))<sub>2</sub>: 171 p,<sup>[20b]</sup> Gd<sub>2</sub>(DOPTA): 200 ps,<sup>[8a]</sup> Gd<sub>2</sub>CS(DO3A-PNBn)<sub>2</sub>: 183 ps,<sup>[8c]</sup> Gd<sub>2</sub>bipy(DO3A)<sub>2</sub>: 185 ps.<sup>[28b]</sup>

A rotational correlation time  $^{298}\tau_R^{\text{mono}}$  of “only”  $\sim 130$  ps can be explained by the rod-like shape of the binuclear compounds (Figure 4 and Figure 6). The rotational motion sensed by the Gd-H vector is probably close to the rotation around the Gd-Gd axis (Figure 4); rotation around an axis perpendicular to the Gd-Gd axis would lead to much slower  $^{298}\tau_R^{\text{mono}}$ .

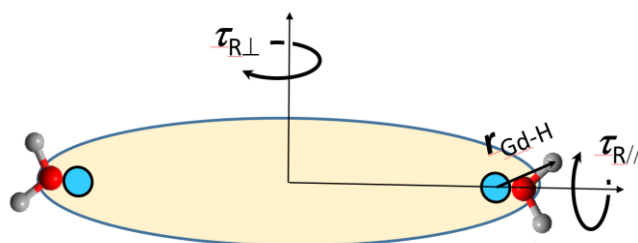


Figure 4. Anisotropic rotation of binuclear compounds.

The rotational correlation times for the aggregates are  $^{298}\tau_R^{\text{agg}} \approx 2.6$  ns for GdL1 and  $\approx 4$  ns for the binuclear compounds with relatively big statistical errors. The increase by more than a factor of twenty from a monomer to essentially a dimer seems to be quite substantial. An effective molecular radius of the aggregates can be estimated to  $r_{\text{eff}} \approx 1.5$  nm using the Debye-Stokes equation (equation 7). Such a radius is fully compatible with the elongated molecular mechanics structures (see below). The strong increase from  $^{298}\tau_R^{\text{mono}} \approx 0.13$  ns to  $^{298}\tau_R^{\text{agg}} \approx 4$  ns can therefore be explained by the anisotropic rotation of the monomer with  $r_{\text{GdH}}$  sensing the fast motion and the more isotropic rotation of the aggregates with  $r_{\text{GdH}}$  sensing the slow overall motion. This explanation implies the absence of fast internal motion in the aggregates (for more detailed information see Supporting Information).

$$\tau_R = \frac{4\pi\eta r_{\text{eff}}^3}{3k_B T} \quad (7)$$

The NMR relaxation results clearly show that the binuclear but also the mononuclear compounds form weak aggregates in solution. Only 15 % in the binuclear compounds and 10 % in the mononuclear compound of the total amount of Gd(III) is present in aggregates. This is however enough to influence markedly the NMRD profiles (Figure 5c).

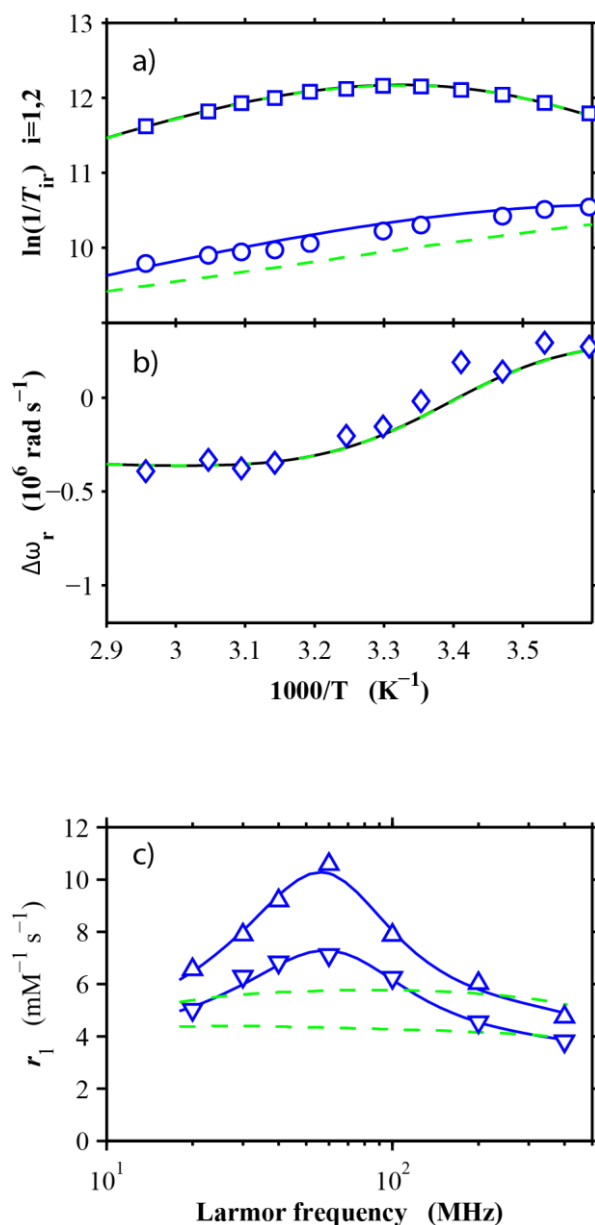


Figure 5. Influence of aggregation of Gd<sub>2</sub>L<sub>3</sub> on <sup>17</sup>O NMR and <sup>1</sup>H NMRD: blue full curves calculated with parameters in Table 1; green dashed curves calculated with 100% monomer. (a) <sup>17</sup>O  $1/T_{2r}$  (□) and  $1/T_{1r}$  (○); (b) <sup>17</sup>O  $\Delta\omega_r$  (◇); (c) <sup>1</sup>H NMRD at 25 °C (△) and 37 °C (▽).

The relaxivities of the binuclear compounds Gd<sub>2</sub>L<sub>2</sub> and Gd<sub>2</sub>L<sub>3</sub> are about 50% higher than that of the mononuclear GdL<sub>1</sub>: at 60 MHz and 37 °C the relaxivities of Gd<sub>2</sub>L<sub>2</sub> and Gd<sub>2</sub>L<sub>3</sub> are  $r_1 = 7.3$  mM<sup>-1</sup>s<sup>-1</sup> and  $r_1 = 7.1$  mM<sup>-1</sup>s<sup>-1</sup> respectively, and GdL<sub>1</sub> has  $r_1 = 4.7$  mM<sup>-1</sup>s<sup>-1</sup>. The relaxivity of the mononuclear compound is slightly higher than that of Gd(DOTA) ( $r_1 = 3.1$  mM<sup>-1</sup>s<sup>-1</sup> at 60 MHz, 37 °C<sup>[29]</sup>) which is mainly due to the 10 % of aggregates formed. Without aggregates one can calculate a relaxivity of 3.7 mM<sup>-1</sup>s<sup>-1</sup> at 37 °C and 60 MHz. The remaining small increase is due to the C4 chain attached to the chelate.



## DEER and Modelling Studies

Distributions of Gd–Gd distances for the binuclear chelates were measured with double electron-electron resonance (DEER) technique.<sup>[30]</sup> This pulse EPR technique allows detecting static magnetic dipolar interaction in the pairs of paramagnetic centers, which is then recalculated into the distance distribution. Currently, most applications of DEER technique<sup>[31]</sup> are found in the field of structural biology in combination with site-directed spin labeling,<sup>[32]</sup> but the technique is generally applicable to any large molecules possessing paramagnetic centers with nanometer-range distances between them. While most DEER experiments are done with pairs of nitroxide radicals, over the last seven years gadolinium chelates were proved to be a possible alternative type of spin centers for DEER measurements.<sup>[33]</sup>

We performed Gd-Gd DEER measurements on both Gd<sub>2</sub>L2 and Gd<sub>2</sub>L3 chelates, in order to determine the distribution of distances between the two Gd(III) centers and try to understand if the distances may have influence on the binuclear compounds relaxivity. The DEER experiment is done in a frozen glassy state, and thus can be considered as a static snap-shot of the dynamic ensemble of conformations present in solution. The measured distance distribution is, however, limited to the inter-spin distances above approximately 1.3 nm due to the bandwidth limitation of the microwave pulses. The upper limit of the detectable distances is set by the transverse relaxation time of the paramagnetic centers, and this limit was well beyond the longest detected distances in our experiments.<sup>[34]</sup>

The longest Gd-Gd distances present in the samples were about 2.7 nm for Gd<sub>2</sub>L2 and about 3 nm for Gd<sub>2</sub>L3 (See Supporting Information). In both cases the fitted distance distributions appeared broad and covered the whole range of distances from the corresponding upper distance value down to the shortest detectable distance. Neither for Gd<sub>2</sub>L2 nor for Gd<sub>2</sub>L3 could the intra-molecular DEER form factors be perfectly fitted by the standard routines of DeerAnalysis<sup>[35]</sup> package, suggesting the presence of some Gd-Gd distances below the detection limit. Such short distances are known to cause fitting deviations. Still, an estimate of the fraction of such short distances in the statistical ensemble is very difficult in the present case. Typically the fraction of detected spin pairs is estimated from the depth of dipolar oscillations in the DEER time trace. For short Gd-Gd distances, however, other effects, such as interference with strong pseudo-secular term in the dipolar spin-Hamiltonian, were claimed to influence

DEER modulation depth, example given by a bis-Gd compound, similar to the ones studied in this work.<sup>[36]</sup> We can thus conclude that DEER measurements are in line with the flexible molecular structure of the studied compounds, and that these measurements would not contradict the presence of shorter Gd-Gd distances, originating, for instance, from some degree of aggregation (as no molecular conformations with Gd-Gd distances below 1.3 nm were predicted by modeling, see below). The estimate of the possible degree of aggregation could not be unambiguously done from the given EPR data.

Molecular modelling using MM3 force field in vacuum afforded Gd-Gd distances for Gd<sub>2</sub>L2 and Gd<sub>2</sub>L3 (Figure 6) which are in good agreement with the DEER results. The distances reported for each compound are the longest and the shortest distance from short molecular dynamics sampling. The linker is the only feature that distinguishes the two chelates. 1,3-phenyldiacetate is a lengthier linker than adipate so, the small difference found in the Gd-Gd distances for completely stretched conformation was predictable. The slightly longer rotational correlation time of the monomeric Gd<sub>2</sub>L3 also reflects its lengthier linker.

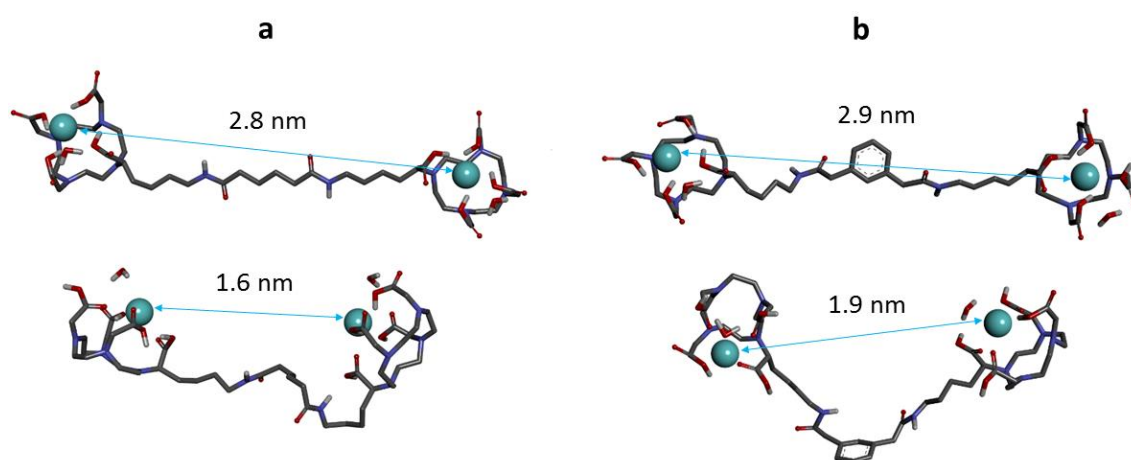


Figure 6. Molecular mechanics structures (MM3 force field) illustrating possible conformations of Gd<sub>2</sub>L2 and Gd<sub>2</sub>L3 and Gd-Gd distances.

## Conclusion

In this work, we developed a synthetic route for the synthesis of DOTA-AHA (L1), a novel DOTA-based bifunctional chelator for trivalent gadolinium, and its derivatives Bis(DOTA-AHA)adipate (L2) and Bis(DOTA-AHA)1,3-phenyldiacetate (L3). The

relaxometric properties of their Gd(III) chelates could only be understood on the basis of a model that envisages the formation of aggregates in solution.

The chelate Gd(DOTA-AHA) (GdL1) has a slightly faster water exchange than Gd(DOTA)<sup>-</sup> but its higher relaxivity is due to the existence of circa 10% of Gd(III) in the aggregated form, besides to the lateral pendant C4-NH<sub>2</sub> chain. The binuclear derivatives [Gd<sub>2</sub>(Bis(DOTA-AHA)adipate)]<sup>2-</sup> (Gd<sub>2</sub>L2) and [Gd<sub>2</sub>(Bis(DOTA-AHA)1,3-phenyldiacetate)]<sup>2-</sup> (Gd<sub>2</sub>L3) do not show a considerable relaxivity improvement in comparison to other existing DO3A-based binuclear Gd(III) chelates for lower frequencies (20 MHz), despite the higher water exchange rates on DOTA based chelates in comparison to DO3A based chelates. However, these binuclear chelates show a considerable augment in relaxivity as the frequency increases, surpassing other Gd(III) binuclear constructs at the frequencies with relevance for MRI scans (between 50 and 100 MHz). This augment in relaxivity has also been attributed to aggregation of the chelates (with approximately 15% of Gd(III) in the aggregated form).

For the binuclear chelates, DEER and modelling studies afforded the distances of maximum and minimum approach of the two Gd(III) centers in a very reasonable agreement, consistent with the assumption that chelates of such dimensions by themselves, without aggregation, would not present such high relaxivities.

## Experimental

### Chemicals and Materials

Analytical grade solvents were used and dried by the usual methods when was needed. Analytical grade reagents were purchased from Sigma-Aldrich, Acros, Bachem, Merck, Chematech and used without further purification. <sup>17</sup>O-enriched water was purchased from IsoTrade GmbH (Mönchengladbach, Germany).

The reactions were monitored by thin layer chromatography (TLC) on glass plates coated with silica gel 60 F<sub>254</sub> (Whatman) and detection was made by examination under UV light (240 nm), by adsorption of iodine vapor and/or by spraying with ninhydrin. Chromatographic separations were performed on silica gel 60 (Whatman 230-240 Mesh).

### Instruments

The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (assigned by DEPT, HSQC and HMBC techniques) were recorded on a Bruker Avance III 400 spectrometer, operating at 400.13 MHz and 100.62 MHz, for  $^1\text{H}$  and  $^{13}\text{C}$  NMR respectively. The chemical shifts ( $\delta$ ) are reported in ppm, relative to TMS (tetramethylsilane) for  $\text{CDCl}_3$  solvent ( $^1\text{H}$ ,  $\delta=7.26$ ;  $^{13}\text{C}$ ,  $\delta=77.16$ ) or DMSO solvent ( $^1\text{H}$ ,  $\delta=2.50$ ;  $^{13}\text{C}$ ,  $\delta=39.52$ ), and relative to TSP (3-(trimethylsilyl)propionic-2,2,3,3- $\text{d}_4$  acid sodium salt) for  $\text{D}_2\text{O}$  solvent ( $^1\text{H}$ ,  $\delta=4.79$ ).<sup>[37]</sup> The high resolution mass spectra (HRMS) were recorded on a VG Autospec M spectrometer. The pH measurements were performed on a pH meter Crison micro TT 2050 with an electrode Mettler Toledo InLab 422.

The proton longitudinal relaxation rates ( $1/T_1$ ) for the water nuclear magnetic relaxation dispersion profiles (NMRD) were measured using the following equipment: Bruker minispecs mq20 0.47 T ( $^1\text{H}$  Larmor frequency: 20 MHz); mq30 0.70 T (30 MHz); mq40 0.94 T (40 MHz); and mq60 1.41 T (60 MHz); Bruker Avance console connected to 2.35 T (100 MHz) and 4.7 T (200 MHz) cryomagnets and Bruker Avance II 9.4 T (400 MHz). The temperature was controlled either by a thermostated gas flow (cryomagnets) or by pumping a thermostated liquid through the probe (minispecs). All temperatures were measured by substitution technique.<sup>[38]</sup> The variable-temperature  $^{17}\text{O}$  measurements were performed on a Bruker Avance II 9.4 T ( $^{17}\text{O}$  Larmor frequency: 54.3 MHz) spectrometer, equipped with a Bruker BVT3000 temperature control unit and a Bruker BCU05 cooling unit. The susceptibility measurements were performed on a Bruker Avance 400 spectrometer, also equipped with a BVT-3000 temperature control unit.

For the EPR experiments, the Gd(III)-Gd(III) distance measurements were performed with the 4 pulse DEER experiment<sup>[30b, 33a]</sup> at Q band on a home-built high microwave power spectrometer<sup>[39]</sup> equipped with a rectangular resonator accommodating for 3 mm outer diameter samples.<sup>[40]</sup> The temperature was set and stabilized with a He-flow cryostat Oxford Instruments ER 4118 CF.

## Synthesis

**6-Amino(Alloc)-2-bromohexanoate benzhydryl ester (compound 1 and 2).** 3.965 g (11.52 mmol) of Lys(Alloc)-OH and 4.148 g (40.3 mmol) of sodium bromide were dissolved in 23.05 mL (23.05 mmol) of 1 M hydrobromic acid and the solution was

cooled down in an ice bath. 1.589 g (23.03 mmol) of sodium nitrite were added in small portions and the mixture was stirred for 2 hours at 0 °C. 45 mL of water and 105 mL of ethyl ether were added to the mixture. The organic phase was collected, and the aqueous phase was extracted with 3x35 mL of ethyl ether. The organic phases were combined, washed with 3x35 mL of brine, dried with anhydrous MgSO<sub>4</sub> and concentrated under reduced pressure to give a yellow oil. The oil was dissolved with 90 mL of acetone, stirred at 0 °C, and 2.684 g (13.82 mmol) of DDM dissolved in 150 mL of acetone were added dropwise over a period of 1 hour. The mixture was stirred for 4 hours at 0 °C and over 2 days at room temperature. The solution was concentrated under reduced pressure to give a yellow oil. The oil was purified by column chromatography over silica gel 60 (cyclohexane/ethyl acetate 4:1) to afford 5.301 g (52.3%) of a yellow oil. <sup>1</sup>H NMR (400 MHz, DMSO, TMS, δ): 1.16-1.47 (4H, m, γ-CH<sub>2</sub> + δ-CH<sub>2</sub>), 1.85-2.08 (2H, m, β-CH<sub>2</sub>), 2.92 (2H, q, J=6.3Hz, ε-CH<sub>2</sub>), 4.43 (2H, d, J=5.6Hz, OCH<sub>2</sub>(Alloc)), 4.69 (1H, t, J=7.2Hz, α-CH), 5.14 (1H, dd, J<sub>1</sub>=10.4Hz, J<sub>2</sub>=1.6Hz, CH-cis(Alloc)), 5.25 (1H, dd, J<sub>1</sub>=17.2Hz, J<sub>2</sub>=2.0Hz, CH-trans(Alloc)), 5.83-5.91 (1H, m, CH(Alloc)), 6.83 (1H, s, CH(Benzhydryl)), 7.16 (1H, t, J=5.8Hz, RNHR'), 7.26-7.43 (10H, m, CH-Ph<sub>2</sub>(Benzhydryl)) ppm. <sup>13</sup>C NMR (100 MHz, DMSO, TMS, δ): 23.28 (γ-CH<sub>2</sub>), 28.58 (δ-CH<sub>2</sub>), 33.90 (β-CH<sub>2</sub>), 39.86 (ε-CH<sub>2</sub>), 46.71 (α-CH), 64.09 (OCH<sub>2</sub>(Alloc)), 77.68 (CH(Benzhydryl)), 116.81 (CH<sub>2</sub>(Alloc)), 126.47 (CH-Ph), 127.96 (CH-Ph), 128.55 (CH-Ph), 133.84 (CH(Alloc)), 139.80 (C-Ph), 155.88 (RCOR'(Alloc)), 168.15 (RCOR'(Benzhydryl)) ppm. HRMS (ESI<sup>+</sup>) – m/z: calculated for C<sub>23</sub>H<sub>26</sub>BrNO<sub>4</sub> (MH<sup>+</sup>) = 460.11235, 462.11030, (MNa<sup>+</sup>) = 482.09429, 484.09224; obtained = 460.11168, 462.10987 (MH<sup>+</sup>), 482.09549, 484.09181 (MNa<sup>+</sup>).

**1,4,7,10-Tetraazacyclododecane-1-(6-amino(Alloc)hexanoate benzhydryl ester) (compound 3).** 725.8 mg (4.37 mmol) of 1,4,7,10-tetraazacyclododecane were dissolved in 70 mL of MeCN and to this solution, 431.7 mg (3.12 mmol) of potassium carbonate were added. 1.438 g (3.12 mmol) of 6-amino(Alloc)-2-bromohexanoate benzhydryl ester dissolved in 40 mL of MeCN were added dropwise over a period of 2 hours. The mixture was stirred overnight at room temperature, filtered under vacuum and concentrated under reduced pressure to give a yellow oil. The oil was purified by column chromatography over silica gel 60 (DCM/EtOH 7:3) to afford 1.608 g (94.2%) of a yellow oil. <sup>1</sup>H NMR (400 MHz, DMSO, TMS, δ): 1.17-1.32 (2H, m, γ-CH<sub>2</sub>), 1.33-

1.51 (2H, m,  $\delta$ -CH<sub>2</sub>), 1.52-1.74 (2H, m,  $\beta$ -CH<sub>2</sub>), 2.52-2.82 (16H, m, 8xCH<sub>2</sub>(cyclen)), 2.92-3.02 (2H, m,  $\epsilon$ -CH<sub>2</sub>), 3.45-3.51 (1H, m,  $\alpha$ -CH), 4.43 (2H, d, J=5.2Hz, OCH<sub>2</sub>(Alloc)), 5.15 (1H, dd, J<sub>1</sub>=9.6Hz, J<sub>2</sub>=2.8Hz, CH-cis(Alloc)), 5.25 (1H, dd, J<sub>1</sub>=17.6Hz, J<sub>2</sub>=1.6Hz, CH-trans(Alloc)), 5.83-5.93 (1H, m, CH(Alloc)), 6.82 (1H, s, CH(Benzhydryl)), 7.16 (1H, t, J=5.4Hz, RNHR'), 7.25-7.45 (10H, m, CH-Ph<sub>2</sub>(Benzhydryl)) ppm. <sup>13</sup>C NMR (100 MHz, DMSO, TMS,  $\delta$ ): 23.20 ( $\gamma$ -CH<sub>2</sub>), 28.90 ( $\delta$ -CH<sub>2</sub>), 29.12 ( $\beta$ -CH<sub>2</sub>), 40.24 ( $\epsilon$ -CH<sub>2</sub>), 44.88, 46.36, 47.92, 48.44 (CH<sub>2</sub>(cyclen)), 64.10 (OCH<sub>2</sub>(Alloc)), 64.46 ( $\alpha$ -CH), 76.70 (CH(Benzhydryl)), 116.86 (CH<sub>2</sub>(Alloc)), 126.70 (CH-Ph), 127.80 (CH-Ph), 128.54 (CH-Ph), 133.83 (CH(Alloc)), 140.46 (C-Ph), 155.90 (RCOR'(Alloc)), 171.81 (RCOR'(Benzhydryl)) ppm. HRMS (ESI<sup>+</sup>) – m/z: calculated for C<sub>31</sub>H<sub>45</sub>N<sub>5</sub>O<sub>4</sub> (MH<sup>+</sup>) = 552.35498; obtained = 552.35443 (MH<sup>+</sup>).

**1,4,7,10-Tetraazacyclododecane-1-(6-amino(Alloc)hexanoate benzhydryl ester)-4,7,10-triacetate *tert*-butyl ester (compound 4).** 1.585 g (2.87 mmol) of 1,4,7,10-tetraazacyclododecane-1-(6-amino(Alloc)hexanoate benzhydryl ester) were dissolved in 150 mL of MeCN and to this solution, 2.383 g (17.24 mmol) of potassium carbonate and 1.36 mL (9.21 mmol) of *tert*-butyl bromoacetate were added. The mixture was stirred overnight at room temperature, filtered under vacuum and concentrated under reduced pressure to give a brown oil. The oil was purified by column chromatography over silica gel 60 (DCM/EtOH 9:1) to afford 2.00 g (77.7%) of a yellow solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, TMS,  $\delta$ ): 1.35-1.65 (31H, m<sub>b</sub>,  $\gamma$ -CH<sub>2</sub> +  $\delta$ -CH<sub>2</sub> + 3x3xCH<sub>3</sub>(*t*-Bu)), 1.66-1.84 (2H, m,  $\beta$ -CH<sub>2</sub>), 2.05-3.59 (24H, m<sub>b</sub>, 8xCH<sub>2</sub>(cyclen) +  $\epsilon$ -CH<sub>2</sub> + 3xCH<sub>2</sub>COR), 3.49-3.61 (1H, m,  $\alpha$ -CH), 4.52 (2H, d, J=5.6Hz, OCH<sub>2</sub>(Alloc)), 5.17 (1H, dd, J<sub>1</sub>=10.4Hz, J<sub>2</sub>=1.2Hz, CH-cis(Alloc)), 5.28 (1H, dd, J<sub>1</sub>=17.2Hz, J<sub>2</sub>=1.6Hz, CH-trans(Alloc)), 5.84-5.91 (1H, m, CH(Alloc)), 6.87 (1H, s, CH(Benzhydryl)), 7.23-7.40 (10H, m, CH-Ph<sub>2</sub>(Benzhydryl)) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, TMS,  $\delta$ ): 23.76 ( $\gamma$ -CH<sub>2</sub>), 27.86 (3xCH<sub>3</sub>(*t*-Bu)), 29.62 ( $\delta$ -CH<sub>2</sub>), 30.00 ( $\beta$ -CH<sub>2</sub>), 40.52 ( $\epsilon$ -CH<sub>2</sub>), 44.77, 47.46, 48.16, 48.53 (CH<sub>2</sub>(cyclen)), 55.56, 55.75 (CH<sub>2</sub>COR), 61.49 ( $\alpha$ -CH), 65.30 (OCH<sub>2</sub>(Alloc)), 78.10 (CH(Benzhydryl)), 81.73, 81.88 (CR<sub>3</sub>R'(*t*-Bu)), 117.41 (CH<sub>2</sub>(Alloc)), 126.97 (CH-Ph), 127.21 (CH-Ph), 128.66 (CH-Ph), 133.02 (CH(Alloc)), 139.17 (C-Ph), 156.36 (RCOR'(Alloc)), 172.78 (RCOR'(*t*-Bu)), 175.09 (RCOR'(Benzhydryl)) ppm. HRMS (ESI<sup>+</sup>) – m/z: calculated for C<sub>49</sub>H<sub>75</sub>N<sub>5</sub>O<sub>10</sub> (MH<sup>+</sup>) = 894.55922; obtained = 894.55867 (MH<sup>+</sup>).

**1,4,7,10-Tetraazacyclododecane-1-((6-amino)hexanoate benzhydryl ester)-4,7,10-triacetate *tert*-butyl ester (compound 5).** 965.0 mg (1.08 mmol) of 1,4,7,10-tetraazacyclododecane-1-(6-amino(Alloc)hexanoate benzhydryl ester)-4,7,10-triacetate *tert*-butyl ester were dissolved in 50 mL of DCM and to this solution, 634.6 mg (10.77 mmol) of borane dimethylamine complex and 1.36 mg (0.11 mmol) of tetrakis(triphenylphosphine)palladium(0) were added. The solution was stirred for 2 hours at room temperature and silica gel 60 was added. The mixture was stirred for 30 minutes at room temperature, concentrated under reduced pressure to give a brown powder. The powder was purified by column chromatography over silica gel 60 (DCM/EtOH 8:2, followed by DCM/EtOH 7:3) to afford 671.5 mg (76.8%) of a brown solid, containing some residues of borane dimethylamine and degraded tetrakis(triphenylphosphine)palladium(0). The product was carried through without further purification. <sup>1</sup>H NMR (400 MHz, DMSO, TMS, δ): 1.32-1.85 (33H, m<sub>b</sub>, β-CH<sub>2</sub> + γ-CH<sub>2</sub> + δ-CH<sub>2</sub> + 3x3xCH<sub>3</sub>(*t*-Bu)), 1.90-3.62 (25H, m<sub>b</sub>, 8xCH<sub>2</sub>(cyclen) + α-CH + ε-CH<sub>2</sub> + 3xCH<sub>2</sub>COR), 6.81 (1H, s, CH(Benzhydryl)), 7.25-7.45 (10H, m, CH-Ph<sub>2</sub>(Benzhydryl)) ppm. <sup>13</sup>C NMR (100 MHz, DMSO, TMS, δ): 23.61 (γ-CH<sub>2</sub>), 25.74 (δ-CH<sub>2</sub>), 27.31 (β-CH<sub>2</sub>), 27.53 (3xCH<sub>3</sub>(*t*-Bu)), 38.58 (ε-CH<sub>2</sub>), 44.18, 47.22, 47.78, 52.27 (CH<sub>2</sub>(cyclen)), 55.23 (CH<sub>2</sub>COR), 60.96 (α-CH), 77.56 (CH(Benzhydryl)), 81.31 (CR<sub>3</sub>R'(*t*-Bu)), 126.64 (CH-Ph), 128.06 (CH-Ph), 128.76 (CH-Ph), 140.47 (C-Ph), 172.67 (RCOR'(*t*-Bu)), 174.47 (RCOR'(Benzhydryl)) ppm. HRMS (ESI<sup>+</sup>) – m/z: calculated for C<sub>45</sub>H<sub>71</sub>N<sub>5</sub>O<sub>8</sub> (MH<sup>+</sup>) = 810.53809; obtained = 810.53754 (MH<sup>+</sup>).

**DOTA-AHA (ligand 1).** 150.0 mg (0.19 mmol) of 1,4,7,10-tetraazacyclododecane-1-((6-amino)hexanoate benzhydryl ester)-4,7,10-triacetate *tert*-butyl ester were dissolved in 3 mL of DCM and 3 mL of TFA. The solution was stirred overnight at room temperature, concentrated under reduced pressure to give a purple oil. The oil was washed 2 times with n-hexane and 2 times with water to give a yellow oil. The oil was dissolved in 15 mL of water and the aqueous solution was washed with 4x10 mL of DCM and concentrated under reduced pressure to afford 105.8 mg of a yellow solid in trifluoroacetate salt form. <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O, TSP, δ): 1.37-1.95 (6H, m<sub>b</sub>, β-CH<sub>2</sub> + γ-CH<sub>2</sub> + δ-CH<sub>2</sub>), 2.75-4.35 (25H, m<sub>b</sub>, 8xCH<sub>2</sub>(cyclen) + α-CH + ε-CH<sub>2</sub> + 3xCH<sub>2</sub>COR) ppm. <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O, TSP, δ): 22.78 (γ-CH<sub>2</sub>), 26.35 (δ-CH<sub>2</sub>), 28.52 (β-

CH<sub>2</sub>), 38.77 ( $\epsilon$ -CH<sub>2</sub>), 50.03, 51.12, 53.21, 55.14 (CH<sub>2</sub>(cyclen)), 59.81 ( $\alpha$ -CH), 63.57 (CH<sub>2</sub>CO<sub>2</sub>H), 169.51 (RCO<sub>2</sub>H), 174.53 (RCO<sub>2</sub>H) ppm. HRMS (ESI<sup>+</sup>) – m/z: calculated for C<sub>20</sub>H<sub>37</sub>N<sub>5</sub>O<sub>8</sub> (MH<sup>+</sup>) = 476.27204; obtained = 476.27149 (MH<sup>+</sup>).

**Bis(1,4,7,10-tetraazacyclododecane-1-((6-amino)hexanoate benzhydryl ester)-4,7,10-triacetate *tert*-butyl ester)adipate (compound 6).** 335.0 mg (413.5  $\mu$ mol) of 1,4,7,10-tetraazacyclododecane-1-((6-amino)hexanoate benzhydryl ester)-4,7,10-triacetate *tert*-butyl ester were dissolved in 15 mL of dry MeCN and to this solution, 25.2 mg (172.3  $\mu$ mol) of adipic acid, 72  $\mu$ L (370.3  $\mu$ mol) of DIPEA, 55.9 mg (413.5  $\mu$ mol) of HOBt and 157.2 mg (413.5  $\mu$ mol) of HATU were added. The solution was stirred for 72 hours at room temperature and more 55.9 mg (413.5  $\mu$ mol) of HOBt and 157.2 mg (413.5  $\mu$ mol) of HATU were added. The solution was stirred for 48 hours at room temperature and more 55.9 mg (413.5  $\mu$ mol) of HOBt and 157.2 mg (413.5  $\mu$ mol) of HATU were added. The solution was stirred for more 48 hours at room temperature and concentrated under reduced pressure to give a white solid. The solid was dissolved in 80 mL of ethyl acetate, and the organic phase was washed with 3x40 mL of 1 M KHSO<sub>4</sub> aqueous solution, 3x40 mL of 1 M NaHCO<sub>3</sub> aqueous solution, and 3x40 mL of brine. The organic phases were combined, dried with anhydrous MgSO<sub>4</sub> and concentrated under reduced pressure to afford 286.1 mg (95.9%) of a white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, TMS,  $\delta$ ): 1.41-1.66 (66H, m<sub>b</sub>, 2x $\gamma$ -CH<sub>2</sub> + 2x $\delta$ -CH<sub>2</sub> + 6x3xCH<sub>3</sub>(*t*-Bu) + 2xCH<sub>2</sub>(linker)), 1.67-1.83 (4H, m, 2x $\beta$ -CH<sub>2</sub>), 1.97-3.40 (52H, m<sub>b</sub>, 16xCH<sub>2</sub>(cyclen) + 2x $\epsilon$ -CH<sub>2</sub> + 6xCH<sub>2</sub>COR + 2xCOCH<sub>2</sub>(linker)), 3.58-3.65 (2H, m, 2x $\alpha$ -CH<sub>2</sub>), 6.35 (2H, m, 2xRNHR'), 6.88 (2H, s, 2xCH(Benzhydryl)), 7.23-7.38 (20H, m, 2xCH-Ph<sub>2</sub>(Benzhydryl)) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, TMS,  $\delta$ ): 24.63 ( $\gamma$ -CH<sub>2</sub>), 26.43 (CH<sub>2</sub>(linker)), 27.86 (3xCH<sub>3</sub>(*t*-Bu)), 29.21 ( $\delta$ -CH<sub>2</sub>), 29.65 ( $\beta$ -CH<sub>2</sub>), 35.27 (COCH<sub>2</sub>(linker)), 38.91 ( $\epsilon$ -CH<sub>2</sub>), 44.38, 47.36, 47.84, 48.21 (CH<sub>2</sub>(cyclen)), 55.59 (CH<sub>2</sub>COR), 61.29 ( $\alpha$ -CH), 78.14 (CH(Benzhydryl)), 81.93 (CR<sub>3</sub>R'(*t*-Bu)), 126.95 (CH-Ph), 128.12 (CH-Ph), 128.63 (CH-Ph), 139.24 (C-Ph), 172.87 (RCOR'(*t*-Bu)), 173.57 (RCOR'), 175.26 (RCOR'(Benzhydryl)) ppm. HRMS (ESI<sup>+</sup>) – m/z: calculated for C<sub>96</sub>H<sub>148</sub>N<sub>10</sub>O<sub>18</sub> (MH<sup>+</sup>) = 1731.10849, (MH<sub>2</sub><sup>2+</sup>) = 866.05816; obtained = 1731.10778 (MH<sup>+</sup>), 866.05753 (MH<sub>2</sub><sup>2+</sup>).



**Bis(1,4,7,10-tetraazacyclododecane-1-((6-amino)hexanoate benzhydryl ester)-4,7,10-triacetate *tert*-butyl ester)1,3-phenyldiacetate (compound 7).** 425.0 mg of (524.6  $\mu\text{mol}$ ) of 1,4,7,10-tetraazacyclododecane-1-((6-amino)hexanoate benzhydryl ester)-4,7,10-triacetate *tert*-butyl ester were dissolved in 20 mL of dry MeCN and to this solution, 42.5 mg (218.9  $\mu\text{mol}$ ) of 1,3-phenyldiacetic acid, 92  $\mu\text{L}$  (524.6  $\mu\text{mol}$ ) of DIPEA, 70.9 mg (524.6  $\mu\text{mol}$ ) of HOBt and 199.5 mg (524.6  $\mu\text{mol}$ ) of HATU were added. The solution was stirred for 72 hours at room temperature and more 70.9 mg (524.6  $\mu\text{mol}$ ) of HOBt and 199.5 mg (524.6  $\mu\text{mol}$ ) of HATU were added. The solution was stirred for 48 hours at room temperature and more 70.9 mg (524.6  $\mu\text{mol}$ ) of HOBt and 199.5 mg (524.6  $\mu\text{mol}$ ) of HATU were added. The solution was stirred for more 48 hours at room temperature and concentrated under reduced pressure to give a yellow solid. The solid was dissolved in 100 mL of ethyl acetate, and the organic phase was washed with 3x50 mL of 1 M  $\text{KHSO}_4$  aqueous solution, 3x50 mL of 1 M  $\text{NaHCO}_3$  aqueous solution, and 3x50 mL of brine. The organic phases were combined, dried with anhydrous  $\text{MgSO}_4$  and concentrated under reduced pressure to afford 334.9 mg (86.1%) of a yellow solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , TMS,  $\delta$ ): 1.17-1.58 (62H,  $m_b$ ,  $2x\gamma\text{-CH}_2 + 2x\delta\text{-CH}_2 + 6x3x\text{CH}_3(t\text{-Bu})$ ), 1.63-1.77 (4H,  $m$ ,  $2x\beta\text{-CH}_2$ ), 1.94-3.39 (48H,  $m_b$ ,  $16x\text{CH}_2(\text{cyclen}) + 2x\epsilon\text{-CH}_2 + 6x\text{CH}_2\text{COR}$ ), 3.49 (4H,  $s$ ,  $2x\text{CH}_2(\text{linker})$ ), 3.51-3.59 (2H,  $m$ ,  $2x\alpha\text{-CH}_2$ ), 6.27 (2H,  $m$ ,  $2x\text{RNHR}'$ ), 6.85 (2H,  $s$ ,  $2x\text{CH}(\text{Benzhydryl})$ ), 7.14-7.40 (24H,  $m_b$ ,  $2x\text{CH-Ph}_2(\text{Benzhydryl}) + 4x\text{CH}(\text{linker})$ ) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , TMS,  $\delta$ ): 24.14 ( $\gamma\text{-CH}_2$ ), 26.38 ( $\delta\text{-CH}_2$ ), 27.85 ( $3x\text{CH}_3(t\text{-Bu})$ ), 29.19 ( $\beta\text{-CH}_2$ ), 39.11 ( $\epsilon\text{-CH}_2$ ), 43.51 ( $\text{CH}_2(\text{linker})$ ), 44.34, 47.31, 47.81, 48.19 ( $\text{CH}_2(\text{cyclen})$ ), 55.68 ( $\text{CH}_2\text{COR}$ ), 61.26 ( $\alpha\text{-CH}$ ), 78.10 ( $\text{CH}(\text{Benzhydryl})$ ), 81.91 ( $\text{CR}_3\text{R}'(t\text{-Bu})$ ), 126.92 ( $\text{CH-Ph}$ ), 127.66 ( $\text{CH-Ph}(\text{linker})$ ), 128.13 ( $\text{CH-Ph}$ ), 128.65 ( $\text{CH-Ph}$ ), 128.80 ( $\text{CH-Ph}(\text{linker})$ ), 130.48 ( $\text{CH-Ph}(\text{linker})$ ), 136.02 ( $\text{C-Ph}(\text{linker})$ ), 139.22 ( $\text{C-Ph}$ ), 171.38 ( $\text{RCOR}'$ ), 172.85 ( $\text{RCOR}'(t\text{-Bu})$ ), 175.18 ( $\text{RCOR}'(\text{Benzhydryl})$ ) ppm. HRMS ( $\text{ESI}^+$ ) –  $m/z$ : calculated for  $\text{C}_{100}\text{H}_{148}\text{N}_{10}\text{O}_{18} (\text{MH}_2^{2+}) = 890.05816$ ; obtained = 890.05753 ( $\text{MH}^{2+}$ ).

**Bis(DOTA-AHA)adipate (ligand 2).** 279.0 mg (161.2  $\mu\text{mol}$ ) of bis(1,4,7,10-tetraazacyclododecane-1-((6-amino)hexanoate benzhydryl ester)-4,7,10-triacetate *tert*-butyl ester)adipate were dissolved in 5 mL of DCM and 5 mL of TFA. The solution was stirred overnight at room temperature and concentrated under reduced pressure to give a purple oil. The oil was washed 2 times with *n*-hexane and 2 times with water to give a

yellow oil. The oil was dissolved in 60 mL of water and the aqueous solution was washed with 4x30 mL of DCM and concentrated under reduced pressure to give a white solid. The solid was purified by ion change column chromatography over dowex® resin (HO<sup>-</sup> form) to afford 245.0 mg of a white solid in hydrochloride salt form. <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O, TSP, δ): 1.35-2.00 (16H, m, 2xβ-CH<sub>2</sub> + 2xγ-CH<sub>2</sub> + 2xδ-CH<sub>2</sub> + 2xCH<sub>2</sub>(linker)), 2.15-2.35 (4H, m, 2xCOCH<sub>2</sub>(linker)), 2.72-4.45 (54H, m, 16xCH<sub>2</sub>(cyclen) + 2xα-CH + 2xε-CH<sub>2</sub> + 3xCH<sub>2</sub>CO<sub>2</sub>H) ppm. <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O, TSP, δ): 23.61 (γ-CH<sub>2</sub>), 24.81 (2xCH<sub>2</sub>(linker)), 26.48 (δ-CH<sub>2</sub>), 28.14 (β-CH<sub>2</sub>), 35.39 (COCH<sub>2</sub>(linker)), 38.15 (ε-CH<sub>2</sub>), 49.12, 50.57, 53.42, 54.32 (CH<sub>2</sub>(cyclen)), 60.28 (α-CH), 63.41 (CH<sub>2</sub>CO<sub>2</sub>H), 168.99 (RCO<sub>2</sub>H), 174.22 (RCO<sub>2</sub>H), 176.41 (RCOR') ppm. HRMS (ESI<sup>+</sup>) – m/z: calculated for C<sub>46</sub>H<sub>80</sub>N<sub>10</sub>O<sub>18</sub> (MH<sup>+</sup>) = 1061.57303, (MNa<sup>+</sup>) = 1083.55498, (MH<sub>2</sub><sup>2+</sup>) = 531.29043, (MH<sub>3</sub><sup>3+</sup>) = 354.52956; obtained = 1061.57249 (MH<sup>+</sup>), 1083.55443 (MNa<sup>+</sup>), 531.29143 (MH<sub>2</sub><sup>2+</sup>), 354.53011 (MH<sub>3</sub><sup>3+</sup>).

**Bis(DOTA-AHA)1,3-phenyldiacetate (ligand 3).** 334.0 mg (187.8 μmol) of Bis(1,4,7,10-tetraazacyclododecane-1-((6-amino)hexanoate benzhydryl ester)-4,7,10-triacetate tert-butyl ester)1,3-phenyldiacetate were dissolved in 5 mL of DCM and 5 mL of TFA. The solution was stirred overnight at room temperature and concentrated under reduced pressure to give a purple oil. The oil was washed 2 times with n-hexane and 2 times with water to give a yellow oil. The oil was dissolved in 60 mL of water and the aqueous solution was washed with 4x30 mL of DCM, concentrated under reduced pressure to give a white solid. The solid was purified by ion change column chromatography over dowex® resin (HO<sup>-</sup> form) to afford 281.0 mg of a white solid in hydrochloride salt form. <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O, TSP, δ): 1.34-1.87 (16H, m, 2xβ-CH<sub>2</sub> + 2xγ-CH<sub>2</sub> + 2xδ-CH<sub>2</sub>), 2.75-4.29 (54H, m, 16xCH<sub>2</sub>(cyclen) + 2xα-CH + 2xε-CH<sub>2</sub> + 3xCH<sub>2</sub>CO<sub>2</sub>H), 3.55 (4H, s, 2xCH<sub>2</sub>(linker)), 7.11-7.26 (3H, m, 2xCH(linker) + 1xCH(linker)), 7.31-7.38 (1H, m, 1xCH(linker)) ppm. <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O, TSP, δ): 23.64 (γ-CH<sub>2</sub>), 26.43 (δ-CH<sub>2</sub>), 28.11 (δ-CH<sub>2</sub>), 38.72 (ε-CH<sub>2</sub>), 42.32 (CH<sub>2</sub>(linker)), 48.85, 50.55, 53.36, 54.20 (CH<sub>2</sub>(cyclen)), 60.21 (α-CH), 63.36 (CH<sub>2</sub>CO<sub>2</sub>H), 127.84 (CH(linker)), 129.76 (CH(linker)), 129.81 (CH(linker)), 135.74 (C(linker)), 168.79 (RCO<sub>2</sub>H), 174.16 (RCO<sub>2</sub>H), 174.28 (RCOR') ppm. HRMS (ESI<sup>+</sup>) – m/z: calculated for C<sub>50</sub>H<sub>80</sub>N<sub>10</sub>O<sub>18</sub> (MH<sup>+</sup>) = 1109.57303, (MH<sub>2</sub><sup>2+</sup>) = 555.29043; obtained = 1109.57248 (MH<sup>+</sup>), 555.28988 (MH<sub>2</sub><sup>2+</sup>).

## Relaxometric Studies

### Sample Preparation

To an aqueous solution of the ligand, a  $\text{GdCl}_3$  solution in a 1:1 mole ratio (for mononuclear chelate) and in a 1:2 mole ratio (for binuclear chelates) was added dropwise (a slight excess of ligand was used). The pH was adjusted to around 4 with the addition of a 0.01 M NaOH solution and the solution was stirred for 1 hour at 60 °C. The pH was adjusted to 5 with the addition of a 0.01 M NaOH solution and the solution was stirred overnight. The pH was then adjusted to 5.7 and the solution was concentrated under reduced pressure.

In all cases, to the final solution,  $\text{H}_2^{17}\text{O}$  ( $^{17}\text{O} = 20.2\%$ ) was added to obtain a final 2%  $^{17}\text{O}$ -enrichment in order to improve the sensitivity of  $^{17}\text{O}$  NMR measurements. The absence of free metal was checked with xylenol orange.<sup>[41]</sup> The final concentration of Gd(III) was determined by susceptibility measurements in the presence of *t*-butanol.<sup>[42]</sup> The Gd(III) concentration in the samples were: monomer chelate  $\approx 20$  mM and binuclear chelates  $\approx 12$  mM.

### $^1\text{H}$ NMRD

Sample tubes with an outer diameter of 5 mm were used for measurements. The proton longitudinal relaxation rates ( $1/T_1$ ) for the water nuclear magnetic relaxation dispersion profiles (NMRD) were measured at 0.47 T ( $^1\text{H}$  Larmor frequency: 20 MHz), 0.70 T (30 MHz), 0.94 T (40 MHz), 1.41 T (60 MHz), 2.35 T (100 MHz), 4.7 T (200 MHz) and 9.4 T (400 MHz). The longitudinal relaxation rates of six chelates with known concentration were measured at two different temperatures (25 and 37 °C). Acidified water (pH = 3.0) was used as an external reference. The relaxivities  $r_1$  ( $\text{mM}^{-1}\cdot\text{s}^{-1}$ ) were calculated using equation 1 using diamagnetic relaxation contributions  $1/T_{1(d)}$  of  $0.31\text{ s}^{-1}$  (400 MHz) /  $0.40\text{ s}^{-1}$  (20 MHz) for 25 °C and  $0.25\text{ s}^{-1}$  (400 MHz) /  $0.29\text{ s}^{-1}$  (20 MHz) for 37 °C, respectively.

$$r_1 = \frac{1}{[\text{Gd}^{3+}]} \left( \frac{1}{T_1} - \frac{1}{T_{1(d)}} \right), \text{ with } [\text{Gd}^{3+}] \text{ in mM} \quad (8)$$

For full equations see Supplementary Information.

## **<sup>17</sup>O NMR**

The samples were sealed in glass spheres adapted for 10 mm NMR tubes, in order to avoid susceptibility corrections to the chemical shifts.<sup>[43]</sup> Variable-temperature <sup>17</sup>O measurements were performed at 9.4 T (<sup>17</sup>O Larmor frequency: 54.3 MHz). The longitudinal (1/*T*<sub>1</sub>) and transverse (1/*T*<sub>2</sub>) relaxation rates were measured using the inversion-recovery<sup>[44]</sup> and the Carr–Purcell–Meiboom–Gill<sup>[45]</sup> pulse sequences, respectively, and chemical shifts ( $\Delta\omega$ ) were measured at 12 different temperatures in the range from 5 to 65 °C. The reduced relaxation rates *T*<sub>1r</sub> and *T*<sub>2r</sub> and the reduced chemical shift differences  $\Delta\omega_r$ , with respect to a pH 3.0 water reference (2% <sup>17</sup>O-enrichment), were calculated using equations 2 to 4. The number of water molecules in the inner sphere of the complex *q* was fixed to one.

$$\frac{1}{T_{ir}} = \frac{1}{P_M} \left( \frac{1}{T_i} - \frac{1}{T_i^{ref}} \right), \text{ where } i = 1, 2 \quad (9)$$

$$\Delta\omega_r = \frac{1}{P_M} (\omega - \omega^{ref}) \quad (10)$$

$$P_M = \frac{q[M^{n+}]}{55.56} \quad (11)$$

For full equations see Supplementary Information (SI)

## **Data Analysis**

For fits of the <sup>1</sup>H NMRD and <sup>17</sup>O NMR data, a Solomon–Bloembergen-based theory was used<sup>[43b, 46]</sup> supplemented with the Lipari–Szabo free-model approach for the internal rotation.<sup>[23a, 23b]</sup> The simultaneous fits were performed using Visualiseur/Optimiseur<sup>[47]</sup> running on a MATLAB® 8.0 (R2012b) platform.

## **DEER Studies**

### **Sample Preparation**

The chelates were prepared using the same method previously used for the relaxometric studies.

For the measurements, solutions were prepared by dissolving 600  $\mu\text{mol}$  of the chelate in 2 ml of  $\text{H}_2\text{O}$  and then the mixture was centrifuged. A 200  $\mu\text{L}$  aliquot of supernatant was collected and mixed with a 200  $\mu\text{L}$  aliquot of glycerol to obtain the final solution.

## Measurements

The Gd(III)-Gd(III) distance measurements were performed with the 4 pulse double electron-electron resonance (DEER) experiment<sup>[30b, 33a]</sup> at Q band on a home-built high microwave power spectrometer<sup>[39]</sup> equipped with a rectangular resonator accommodating for 3 mm outer diameter samples.<sup>[40]</sup> The measurements were performed at 10 K and the temperature was set and stabilized with a He-flow cryostat Oxford Instruments ER 4118 CF.

In the DEER pulse sequence all microwave pulses were set to duration of 12 ns, first inter-pulse interval of 400 ns and the length of the DEER time trace of 3  $\mu\text{s}$  was set. The frequency difference between pump and detection pulses was set to 300 MHz.

## Data Analysis

The DEER time traces were analyzed with the DeerAnalysis software.<sup>[35]</sup> A model free fit with Tikhonov regularization was performed in each case.

## Molecular Modelling

The molecular modelling has been performed Scigress (Fujitsu) Version 3.1.0. Structures are taken from MD conformational searches and energy minimized, both in vacuum. The force-field used is MM3; Gd(III) has been replaced by Y(III) which has approximately the same ionic radius.

## Abbreviations

<b>Alloc</b>	Allyloxycarbonyl
<b>(Bis(DOTA-AHA)1,3-phenyldiacetate)</b>	Bis(1,4,7,10-tetraazacyclododecane-1-((6-amino)hexanoic)-4,7,10-triacetic acid)1,3-phenyldiacetate
<b>(Bis(DOTA-AHA)1,3-phenyldiacetate)</b>	Bis(1,4,7,10-tetraazacyclododecane-1-((6-amino)hexanoic)-4,7,10-triacetic acid)1,3-phenyldiacetate
<b>Boc</b>	<i>tert</i> -Butyloxycarbonyl

<b>CA</b>	Contrast agent
<b>Cyclen</b>	1,4,7,10-tetraazacyclododecane
<b>DCM</b>	Dichloromethane
<b>DDM</b>	Diphenyldiazomethane
<b>DEER</b>	Double electron-electron resonance
<b>DIPEA</b>	<i>N,N</i> -Diisopropylethylamine
<b>DMF</b>	<i>N,N</i> -Dimethylformamide
<b>DMSO</b>	Dimethyl sulfoxide
<b>DOTA-AHA</b>	1,4,7,10-Tetraazacyclododecane-1-((6-amino)hexanoic)- 4,7,10-triacetic acid
<b>DOTA</b>	1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid
<b>DO3A</b>	1,4,7,10-Tetraazacyclododecane-1,4,7-triacetic acid
<b>EPR</b>	Electron paramagnetic resonance
<b>ESI</b>	Electrospray ionization
<b>EtOH</b>	Ethanol
<b>HATU</b>	<i>O</i> -(7-Azabenzotriazol-1-yl)- <i>N,N,N',N'</i> -tetramethyluronium hexafluorophosphate
<b>HMBC</b>	Heteronuclear multiple bond correlation
<b>HOBt</b>	1-Hydroxybenzotriazole
<b>HRMS</b>	High resolution mass spectrometry
<b>Lys</b>	Lysine
<b>MeCN</b>	Acetonitrile
<b>MRI</b>	Magnetic resonance imaging
<b>NMR</b>	Nuclear magnetic resonance
<b>NMRD</b>	Nuclear magnetic relaxation dispersion
<b>Pd/C</b>	Palladium on carbon
<b>SBM</b>	Solomon-Bloembergen-Morgan theory
<b>SD</b>	Standard deviation
<b>SDSL</b>	Site directed spin labelling
<b>TFA</b>	Trifluoroacetic acid
<b>TLC</b>	Thin layer chromatography
<b>TMS</b>	Tetramethylsilane
<b>TSP</b>	3-(Trimethylsilyl)propionic-2,2,3,3-d <sub>4</sub> acid sodium salt

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